The Infectiousness of Tuberculosis Patients Coinfected with HIV

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Abbreviations: IQR, interquartile range; MDR, multidrug-resistant; SD, standard deviation; TB, tuberculosis; XDR, extensively drug-resistant

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ABSTRACT

Background

The current understanding of airborne tuberculosis (TB) transmission is based on classic 1950s studies in which guinea pigs were exposed to air from a tuberculosis ward. Recently we recreated this model in Lima, Perú, and in this paper we report the use of molecular fingerprinting to investigate patient infectiousness in the current era of HIV infection and multidrug-resistant (MDR) TB.

Methods and Findings

All air from a mechanically ventilated negative-pressure HIV-TB ward was exhausted over guinea pigs housed in an airborne transmission study facility on the roof. Animals had monthly tuberculin skin tests, and positive reactors were removed for autopsy and organ culture for *M. tuberculosis*. Temporal exposure patterns, drug susceptibility testing, and DNA fingerprinting of patient and animal TB strains defined infectious TB patients. Relative patient infectiousness was calculated using the Wells-Riley model of airborne infection. Over 505 study days there were 118 ward admissions of 97 HIV-positive pulmonary TB patients. Of 292 exposed guinea pigs, 144 had evidence of TB disease; a further 30 were tuberculin skin test positive only. There was marked variability in patient infectiousness; only 8.5% of 118 ward admissions by TB patients were shown by DNA fingerprinting to have caused 98% of the 125 characterised cases of secondary animal TB. 90% of TB transmission occurred from inadequately treated MDR TB patients. Three highly infectious MDR TB patients produced 226, 52, and 40 airborne infectious units (quanta) per hour.

Conclusions

A small number of inadequately treated MDR TB patients coinfected with HIV were responsible for almost all TB transmission, and some patients were highly infectious. This result highlights the importance of rapid TB drug-susceptibility testing to allow prompt initiation of effective treatment, and environmental control measures to reduce ongoing TB transmission in crowded health care settings. TB infection control must be prioritized in order to prevent health care facilities from disseminating the drug-resistant TB that they are attempting to treat.

The Editors' Summary of this article follows the references.

Introduction

Seminal experiments demonstrating airborne tuberculosis (TB) transmission by droplet nuclei were performed by Riley and coworkers in the 1950s–1960s [1,2]. Guinea pigs acquired TB by breathing exhaust air from a TB ward. The studies demonstrated TB transmission from a minority of patients, marked variability in patient infectiousness, and reduced infectiousness following initiation of effective chemotherapy [1–3]. These classic studies were recently recreated in Lima, Perú, in the modern era of HIV infection and multidrug-resistant (MDR) TB, and again showed striking variability in patient infectiousness [4].

The strongest predictor of TB patient infectiousness is sputum smear status [5–7]. However, considerable variation exists in TB prevalence amongst contacts of smear-positive patients [5], and the importance of smear-negative transmission has also been demonstrated [8]. Other determinants of infectiousness include lung cavitation and cough frequency [9], and cough-inducing procedures have been associated with extensive transmission [10–12]. Additional factors are likely and may include sputum volume or consistency, and TB strain variables such as ability to survive in the airborne state. Recent studies detected culture-positive cough-generated aerosols in only 25% of new TB patients [13].

The relative infectiousness of MDR versus drug-susceptible TB remains controversial. The patients with drug-susceptible TB studied by Riley were four to eight times more likely to infect guinea pigs than were those with drug-resistant disease [1–3]. Epidemiological studies of contacts of both drug-resistant and drug-susceptible TB infected patients have shown no difference in relative transmissibility [14–16], whilst studies utilising molecular epidemiology have had conflicting results [17–20]. Similarly, the effect of HIV infection on TB infectiousness remains disputed [21]. The beneficial effect of anti-TB chemotherapy on reducing TB infectiousness is well known, but this presupposes that the treatment administered is effective. The inadequate treatment of MDR TB and the emergence of extensively drug-resistant (XDR) TB therefore have important consequences for hospital TB infection control policies.

In this paper we report the results of molecular fingerprinting to determine which patients had infected which guinea pigs in our in vivo air sampling model above a TB ward. We investigate factors associated with TB transmission from this group of HIV-coinfected TB patients with a high prevalence of MDR TB.

Methods

Setting

An airborne infection study facility was constructed on the roof of a TB-HIV ward at Hospital Nacional Dos de Mayo, Lima, Perú, as previously described [4]. All air from two 4-bedded negative-pressure patient rooms passed over guinea pigs in the study facility before being exhausted into the atmosphere. Airflow from the ward and into the animal facility was measured using a capture hood (Alnor, Shoreview, United States) at air injection and extraction vents.

Patients

The ward operated as the hospital TB-HIV service, and patient admission, management, and duration of stay were not influenced by the study. All patients were invited to join the study through written consent. An admission questionnaire recorded daily symptoms including cough, haemoptysis, and fever. Twenty-four-hour sputum collections were made daily for auramine microscopy [22] and TB culture using MODS [23]. For nonconsenting patients, anonymised unlinked information routinely available for ward infection control, including sputum microscopy and treatment, was recorded.

Experimental Protocol

Outbred Peruvian guinea pigs were maintained in quarantine for 1–3 mo. Animals were skin tested monthly as described [4] and induration measured 48 h later. Animals were transferred to the hospital following at least two negative skin tests to ensure freedom from TB. In May 2002, 144 animals began ward air exposure, and 6 mo later, 148 animals were added. Monthly skin tests continued throughout 505 d exposure to ward air, and positive reactors were removed for humane killing and autopsy. Evidence of TB infection was sought in lungs, mediastinal lymph nodes, spleen, and liver. Tissues were homogenised and cultured for TB as described [4]. Forty negative control guinea pigs were maintained separately, breathing fresh air.

Determination of Patient Infectiousness

TB drug-susceptibility testing of animal and patient strains for susceptibility to isoniazid, rifampicin, streptomycin, ethambutol, capreomycin, and ciprofloxacin was performed using the tetrazolium microplate assay [24] and DNA fingerprinting performed using spoligotyping [25]. Linkage of source patient with infected guinea pigs relied upon genotypic (identical spoligotype) and phenotypic (drug-susceptibility pattern) concordance and patient ward occupancy 3–9 wk prior to animal PPD conversion, reflecting TB incubation in these guinea pigs [26]. Individual patient infectiousness was determined using the Wells-Riley airborne infection equation (see Text S1) [27]. Determinants of patient infectiousness were assessed by univariate and multiple logistic regression in which nonsignificant factors were sequentially dropped from the model using SPSS and SAS statistical software [28].

Ethical Approval

The study was approved by the Institutional Review Boards at Hospital Dos de Mayo, Asociación Benéfica PRISMA, Perú, and Imperial College London, Hammersmith Hospital Campus, UK. Animal ethical approval was obtained from the Veterinary Medicine Faculty, Universidad Nacional Mayor San Marcos, Lima, Perú, which supervised all animal work.

Results

Patient Characteristics

There were 185 ward admissions by 161 HIV-positive patients, resulting in 2,667 patient days, comprising 118 admissions of 97 pulmonary TB patients (1,798 [67%] patient days), 33 admissions of 30 extrapulmonary TB patients (609 [23%] patient days), and 34 admissions of 34 TB suspects who subsequently had no laboratory evidence of TB (260 [10%] patient days). Of the 64 extrapulmonary disease patients or TB suspects, 59 able to produce sputum were acid-fast-

bacillus smear and TB culture negative. Median length of stay was 11 d (interquartile range [IQR] 6–21 d). Monthly variations in pulmonary TB patient days according to sputum status are shown in Figure 1A. Of 66 sputum culture–positive pulmonary TB admissions, 35 (53%) were sputum smear positive and 31 (47%) were sputum smear negative.

Pulmonary TB patients composed a heterogeneous group of new and existing TB diagnoses, admitted for diagnosis and treatment, adverse treatment effects, or other complications of TB or HIV infection. All patients were HIV positive, none were taking combination antiretroviral therapy, and $CD4^+$ T cell counts were not available. Twelve pulmonary TB patients had isoniazid- or rifampicin-monoresistant strains (251 [14%] pulmonary TB patient days); 21 patients had confirmed MDR TB (434 [24%] pulmonary TB patient days); and 11 patients had presumed MDR TB (treated empirically for drug-resistant disease due to treatment failure; 143 [8.0%] pulmonary TB patient days). No patients had XDR TB. Thirty-four patients had confirmed drug-susceptible TB (687 [38%] pulmonary TB patient days); and 20 patients had presumed drug-susceptible TB treated empirically without drug-susceptibility results (275 [15%] patient days). Two patients with drug-susceptible TB acquired MDR TB with a new spoligotype pattern, which was not demonstrated to have been acquired on the ward. For MDR TB patients, significantly more patient bed days were accounted for by sputum culture-positive patients than by sputum culture-negative patients; in contrast, for non-MDR TB patients, significantly more patient bed days were accounted for by sputum culture-negative patients than by sputum culture-positive patients (both $p < 0.0001$; Figure 2).

The treatment status of pulmonary TB patients is shown in Table 1. "Optimal" signifies an antibiotic regimen suitable for the TB strain drug resistance pattern, whilst "suboptimal" signifies a regimen less likely to result in cure. The suboptimal treatment category included standard first-line therapy (4 mo of isoniazid, rifampicin, pyrazinamide, and ethambutol followed by 2 mo of rifampicin and isoniazid [29]) administered to MDR TB cases whilst indirect drug susceptibility results were awaited, a process commonly taking 2–3 mo. The suboptimal treatment group also included patients with drug-resistant strains given the now abandoned second-line regimen [29] containing standard first-line drugs with the addition of a single drug, streptomycin, and any first-line or second-line therapy that was delayed because of adverse drug reactions or drug shortages.

Spoligotype results were available for 49 (42%) pulmonary TB admissions, corresponding to 39 (40%) of 97 patients. The remaining admissions were predominantly patients with culture-negative sputum, or those unable to produce sputum. Sixteen culture-positive patients, of whom four were smear positive, had no spoligotyping result, due to laboratory contamination or unwillingness to consent. For the 49 admissions with spoligotyping results, 25 different patterns were observed, none of which corresponded to Beijing strains of *M. tuberculosis*. Figure 1B shows the temporal distribution of patient days accounted for by patients with different spoligotype patterns.

Detection of TB Transmission to Guinea Pigs

No positive PPD skin tests were seen in quarantine (760 tests) or unexposed negative control animals (287 tests). During 505 study days, 292 animals were exposed to ward air, an average of 92 each month. A total of 159 animals developed positive tests ($\geq$ 7.5 mm), 124 were TB culture positive, and a further five had autopsy evidence of pulmonary TB [4]. Nine ward air-exposed PPD-negative animals had evidence of TB, as did six of 25 intercurrent deaths between skin tests [4]. In total, cultures were positive in 135 (46%) of 292 animals. No evidence of TB was found in unexposed negative controls.

Of 135 culture-positive guinea pigs, drug susceptibility and spoligotyping results were available for 125. Of this group 121 (97%) were multidrug resistant, one was isoniazid mono-resistant, and three were fully drug susceptible. Eight different spoligotype patterns were observed. Figure 1C shows the distribution of guinea pig TB infections and corresponding spoligotype patterns according to the monthly skin test when TB was diagnosed. Data from intercurrent deaths are included in the subsequent skin test. There was a large monoclonal TB outbreak in month 10 (spoligotype pattern #7), which continued into months 11 and 12. This strain reappeared in months 14 and 15, but was not seen in month 13.

In ten guinea pigs, spoligotyping was performed on positive cultures from three separately dissected tissues (lymph nodes, lung, spleen). Spoligotype patterns were identical in all tissues. In nine animals with multiple pulmonary primary foci, two foci were dissected individually from different lung lobes with separate sets of instruments. All primary foci yielded identical spoligotype patterns. Distribution of infection according to animal cage location was random, consistent with airborne transmission from the ward, not horizontal spread between animals.

Infectiousness of Patients

Of 125 guinea pigs with spoligotype results, 122 (98%) were matched with a patient with an identical TB drug-susceptibility and spoligotype pattern who had resided on the ward 3–9 wk prior to the guinea pig skin test conversion. Ten different infectious patients were identified, who had seven spoligotype patterns. Of these, one was unique to one patient, and the remainder were seen in multiple patients. However, in the 3–9 wk preceding the infection of each guinea pig cluster, only one patient was ever identified with such a pattern. The characteristics of the ten identified infectious patients are shown in Table 2. Six had MDR, one had monoresistant, and three had drug-susceptible TB. Amongst the six patients with MDR TB strains, five strains were additionally resistant to ethambutol, of which two strains were also resistant to streptomycin. Two of the three patients with drug-susceptible TB were newly started on treatment and hence they had spent time on the ward untreated, and the third had stopped treatment. All identified patients with infectious drug-resistant TB had been suboptimally treated: six were on inadequate treatment regimens, and one had had the initiation of correct treatment delayed for 11 d.

There were at least two further infectious patients. One infected two animals with an MDR TB strain not seen amongst patients' spoligotyping results. During the 3–9 wk prior to the animals' infection there were three sputum culture–positive MDR TB (one smear-positive) patients without spoligotyping results on the ward, any of whom could have infected these animals. A second unidentified infectious

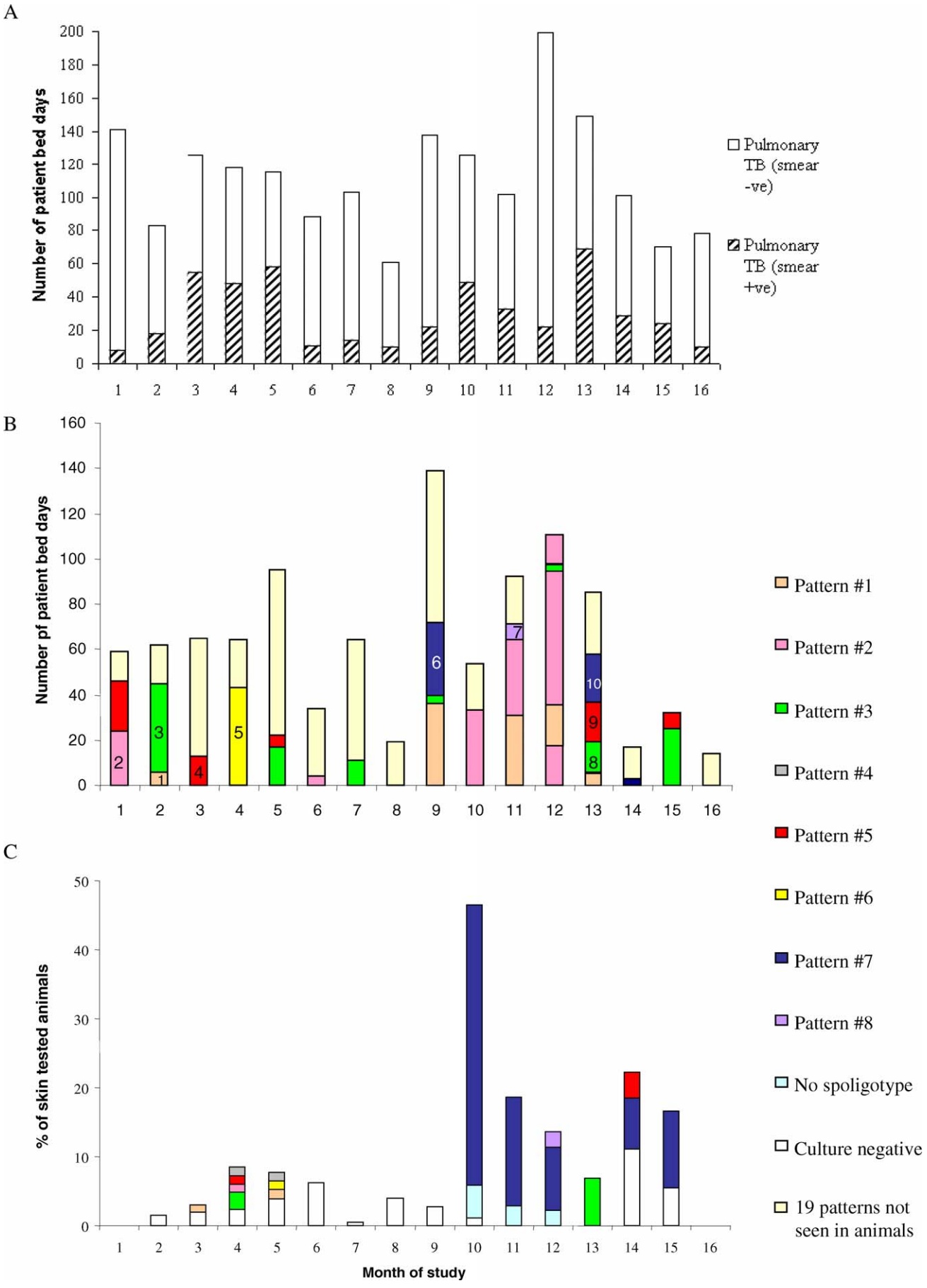
A
Number of patient bed days
Pulmonary TB (smear -ve)
Pulmonary TB (smear +ve)
B
Number pf patient bed days
Pattern #1
Pattern #2
Pattern #3
Pattern #4
Pattern #5
Pattern #6
Pattern #7
Pattern #8
No spoligotype
Culture negative
19 patterns not seen in animals
C
% of skin tested animals
Month of study

Figure 1. Pulmonary TB Patient Bed Days and TB Strain Spoligotype Pattern Compared with TB Infection in Guinea Pigs by Study Month

(A) Number of bed days in each study month resulting from pulmonary TB patients, who were either smear positive (patterned bars, "+ve"), or smear negative (white bars, "−ve") at the time of admission.

(B) Number of bed days in each study month resulting from pulmonary TB patients for whom a TB strain spoligotype pattern was available. Each block of colour corresponds to one patient, and each colour to one of the eight spoligotype patterns observed in the guinea pigs. Pale yellow represents the remaining 19 patterns observed in patients whose TB was not seen in the guinea pigs. If a patient resided on the ward for a period spanning more than one study month, that patient is included in the month where they accounted for more smear positive patient bed days. The coloured blocks containing numbers correspond to the ten identified infectious patients, numbered 1 to 10 in Table 2.

(C) Percentage of animal colony skin tested each study month that were PPD positive. Each colour represents one spoligotype pattern, except for pale blue, which represents ten guinea pigs culture positive for TB but for which spoligotype patterns were unavailable. White represents animals that were PPD positive but TB culture negative. Animals culture positive for TB that were PPD false negatives or that died between skin tests were included in the subsequent month's skin test.

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patient (potentially one of the three above) resulted in a guinea pig PPD conversion seen in month 5, with an MDR TB strain with spoligotype pattern #1.

Table 2 also shows patient infectiousness, q, the number of infectious quanta produced per hour. For absolute ventilation in the Wells-Riley equation, mean total air entering the animal enclosure was used: 28 m^3/min (standard deviation [SD] 0.9 m^3/min). This comprised ward air (21 m^3/min), air from a small room used in the mornings for non-TB patients (3.4 m^3/min), and outside air infiltration into ducts between ward and animal house (3.4 m^3/min). The calculation methodology and a worked example for infectious patient number 6 are provided in Text S1. Average patient infectiousness over the entire study was 8.2 infectious quanta/h (using 174 total animal infections) or 6.7 (using 144 total animal infections with evidence of disease, excluding PPD-positive animals without autopsy or culture evidence of TB).

Determinants of Infectiousness

Regression analysis was performed for putative determinants of infectiousness for pulmonary TB admissions with spoligotype results. Admissions rather than patients were analysed, because patients with multiple admissions changed sputum smear, drug-susceptibility, or treatment status. Forty-nine admissions (39 patients) were included. Ten were classed as infectious resulting in TB transmission to guinea pigs, and 39 as noninfectious. In univariate analysis, the same patient characteristics were significantly associated with both whether or not TB transmission occurred (Table 3) and with the degree of patient infectiousness (q, see Text S1; Table 4): sputum smear-positivity, MDR TB versus non-MDR TB, and suboptimal treatment. Multiple regression analysis of whether or not a patient admission caused TB transmission to guinea pig(s) showed an independent statistically significant association only with MDR status ($p = 0.02$); TB transmission to guinea pigs was also observed more frequently in admissions of sputum smear-positive (versus smear-negative) patients, but this association was not statistically significant ($p = 0.08$; Table 3). Predicted probabilities were estimated from an exact multiple logistic regression: the predicted probability of infectiousness was 3% for smear-negative non-MDR TB patients; 18% for patients who were either smear positive or had MDR TB; and 59% for smear-positive MDR TB patients. Further univariate analyses were performed on a subset of 33 consenting patient admissions for which symptom information such as cough frequency was available, but no variable approached significance (all $p > 0.6$), which may reflect low power due to the small sample.

Multiple regression analysis of data from all patients identified as having caused TB transmission demonstrated that the degree of patient infectiousness was independently significantly associated with MDR TB; patient infectiousness appeared possibly to increase with sputum-smear positivity and days on the ward without treatment, but these associations were not found to be statistically significant (Table 4). This statistical model allowed patient character-

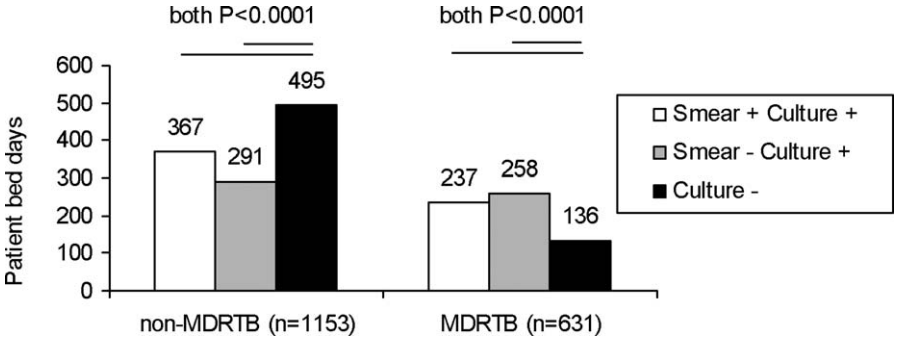

Figure 2. Pulmonary TB Patient Bed Days According to Sputum Smear Status and TB Drug Susceptibility

The numbers of patient bed days accounted for by patients with MDR TB, or with non-MDR TB, are shown. Results are subdivided into sputum-smear positive and culture positive (white shading); sputum-smear negative and culture positive (dark grey shading); and culture negative (black shading). Two patients are not included in this figure: one culture-positive patient with drug-susceptible TB (accounting for 6 patient days) with an unavailable smear result; and one smear-negative, culture-positive patient (accounting for 8 patient days) with no TB drug-susceptibility information. The drug-resistant category included 32 MDR TB cases and 12 isoniazid or rifampicin monoresistant cases. In non-MDR TB admissions, 34 smear-negative culture-positive and 241 culture-negative patient bed days were accounted for by patients treated empirically, without confirmation of drug-susceptibility status in our laboratory. In MDR TB admissions, 45 smear-negative culture-positive and 98 culture-negative patient days were accounted for by such patients. Comparisons between groups were made using the two-sample z-test of proportions.

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Table 1. Antituberculosis Chemotherapy Treatment Status of Confirmed and Presumed Drug-Susceptible and Drug-Resistant Pulmonary TB Admissions to the Ward

Treatment	Category	Drug-Susceptible TB Patient Admissions			Drug-Resistant TB Patient Admissions			All Pulmonary TB Patient Admissions[a]		
		n	Days on Ward	Days without Treatment	*n*	Days on Ward	Days without Treatment	*n*	Days on Ward	Days without Treatment
Established[b]	Optimal	39	492 (27%)	19 (1%)	8	115 (6%)	0	47	607 (34%)	19 (1%)
	Suboptimal	—	—	—	8	115 (6%)	0	8	115 (6%)	0
Recent[c]	Optimal	5	84 (5%)	0	4	100 (6%)	0	9	184 (10%)	0
	Suboptimal	—	—	—	7	159 (9%)	0	7	159 (9%)	0
New[d]	Optimal	19	323 (18%)	77 (4%)	3	47 (3%)	13 (1%)	22	370 (21%)	90 (5%)
	Suboptimal	—	—	—	8	167 (9%)	48 (3%)	8	167 (9%)	48 (3%)
None[e]	—	4	63 (4%)	63 (4%)	9	78 (4%)	78 (4%)	13	141 (8%)	141 (8%)
Unknown	—	—	—	—	3	47 (3%)	—	3	47 (3%)	—
Total	—	**67**	**962 (54%)**	**164 (9%)**	**50**	**828 (46%)**	**139 (8%)**	**117**	**1,790 (100%)**	**298 (17%)**

Numbers of days on the ward are shown, with percentage of total pulmonary TB days (1,798 d) in parenthesis. "Optimal" indicates an antibiotic regimen suitable for the TB strain drug resistance pattern; "suboptimal" indicates a regimen less likely to result in cure.

[a]One patient who resided on the ward for 8 d and for whom no drug-susceptibility information is available has been excluded from this table.

[b]"Established" treatment denotes treatment of $\geq$ 2 wk duration.

[c]"Recent" treatment denotes treatment commenced within the previous 2 wk.

[d]"New" treatment denotes treatment commenced during the study hospital admission.

[e]"None" denotes abandoned treatment, or treatment withdrawn due to adverse effects.

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istics to be used to predict the number of infectious quanta produced per hour (q). The regression model (Table 4) indicated that: non-MDR TB, smear-negative patients with undelayed treatment had a predicted infectiousness $q = 0.3$; patients with MDR TB or sputum-smear positivity without treatment delay had a predicted $q = 3.9$; more than 2 d of treatment delay resulted in an increase in q of 1.7; sputum-smear positive MDR TB had a predicted $q = 24$; MDR TB and (more than 2 d of delayed treatment or sputum-smear positivity) had predicted $q = 14$; and sputum-smear positive MDR TB with more than 2 d of delayed treatment had a predicted $q = 54$.

Discussion

This study provides novel characterization of the heterogeneity and determinants of infectiousness of HIV-positive TB patients by applying molecular strain characterization to track airborne TB transmission to guinea pigs. This research has for the first time (to our knowledge) demonstrated that amongst HIV-positive patients TB infectiousness is extremely variable, that a few HIV-positive patients were highly infectious, and that inadequately treated MDR TB patients accounted for the great majority of TB transmission. In contrast to seminal studies of TB transmission using a similar guinea-pig method of detection half a century ago, this study was conducted in a real-life busy ward in a low-resource setting with unselected patients, composed of a heterogeneous mix of new and established diagnoses of drug-susceptible and drug-resistant TB, with varying treatment regimens. These results therefore have important implications for TB infection control, especially in the era of increasingly integrated TB and HIV care and the emergence of XDR TB strains.

Average patient infectiousness over the whole study period for these HIV-positive TB patients with high rates of MDR TB was up to six times greater than that calculated for the heterogeneous mix of patients in the 1950s studies ($q = 1.25$) [4,27]. However, this average masks considerable variability in infectiousness between patients. Three highly infectious patients were observed, all with MDR TB, with q-values of 40, 52, and 226. It should be noted that these q-values reflect TB transmission from humans to guinea pigs. The infectious dose of *M. tuberculosis* for humans is unknown; hence the concept of infectious quanta used in airborne infection models [27]. For fully virulent *M. tuberculosis* strains just one droplet nucleus can establish infection and disease in guinea pigs, but for strains of reduced virulence for guinea pigs, up to four aerosolised colony-forming units may be required to establish a single pulmonary primary focus [30]. Thus some caution is needed in comparing the infectiousness of patients in this study with published q-values calculated for human-to-human transmission, such as $q = 13$ for an untreated office worker who infected 27 coworkers over 4 wk prior to diagnosis [31], and $q = 250$ for an outbreak associated with intubation and bronchoscopy of a TB patient [11,27]. However, comparisons can be drawn with q-values calculated for Riley's study: $q = 1.25$ average for all patients; $q = 60$ for the most infectious case, with laryngeal TB [27]. Direct comparisons should, however, be made cautiously owing to methodological differences between the studies such as the type of guinea pig used and the cutoff for a positive skin test [32]. This model for identifying infectious patients required TB strains to be not only transmissible to guinea pigs, but also sufficiently virulent to cause disease from which a strain could be recovered with corresponding spoligotype. Animals with positive PPD skin test conversions but culture negative for TB were observed throughout the study. Due to the continuous exposure to ward air, mixed infections might be expected in the animals, but in fact were not observed despite the culture of separately dissected lung foci. Because they are relatively uncommon, mixed infections were not specifically sought in patients [33].

Table 2. Characteristics of Identified Infectious Patients

Patient Number	Days on Ward	Sputum Smear	Drug Susceptibility	Treatment	Days on Ward without Treatment	Haemoptysis	Cough	Fever	Sputum Volume (ml)[a]	Chest X-Ray Cavitation	Number of Guinea Pigs Infected	Spoligotype Pattern Number	Calculated Infectious Quanta per Hour (± 2 SD)
1	6	3+	MDR	Recent suboptimal	0	+	+	+	24	–	1	1	12 (0.8)
2	24	1+	Susceptible	New	3	–	+	–	5	–	1	2	3.0 (0.2)
3[b]	39	2+	MDR	New suboptimal	5	n/a	n/a	n/a	n/a	n/a	2	3	2.9 (0.2)
4	13	2+	Susceptible	New	1	–	+	–	0	–	1	5	5.5 (0.4)
5	43	3+	MDR	Recent suboptimal	0	–	+	+	16	–	1	6	1.8 (0.1)
6	32	3+	MDR	New correct, but delayed	11	–	+	+	50	–	108	7	226 (15)
7	7	3+	Isoniazid resistant	Recent suboptimal	0	–	+	+	8	–	1	8	18 (1.2)
8	13	3+	MDR	Established suboptimal	0	–	+	+	24	–	2	3	40 (2.6)
9[b]	18	Negative	Susceptible	Stopped	18	n/a	n/a	n/a	n/a	n/a	1	5	12 (0.8)
10	21	3+	MDR	Recent suboptimal	10	–	+	+	90	–	4	7	52 (3.4)
11[c]	—	—	MDR	—	—	—	—	—	—	—	2	—	—
12[c]	—	—	MDR	—	—	—	—	—	—	—	1	—	—

Characteristics of the ten infectious patients identified by matching TB spoligotype pattern and temporal association with a guinea pig infection are shown.

[a]Maximum daily sputum volume during ward admission is recorded.

[b]Patients 3 and 9 did not consent and therefore symptom details are unavailable, denoted n/a.

[c]Infectious patients 11 and 12, both with MDR strains, remained unidentified.

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The relative infectiousness of patients in this study was highly variable, and a patient with MDR TB was found to be highly infectious, producing 226 infectious quanta/h. The TB strain responsible, not a Beijing strain, was also seen in a second highly infectious MDR TB patient, producing 52 infectious quanta/h. This observation suggests a potentially strain-related factor involved in transmissibility, perhaps an enhanced ability to survive aerosolisation and the physical stresses of being airborne. An alternative explanation might be an effect on disease phenotype. It is interesting to note that both patients had fever and cough and produced large volumes of sputum, between 10 and 90 ml daily. Neither patient had cavitation on chest X-ray. Although no formal ear-nose-throat assessment was documented, neither was diagnosed with laryngeal TB. Both patients also spent time on the ward untreated. The first was untreated for the first 11 d of a 32 d admission before second-line drugs were commenced, due to difficulties in access to medications. The second patient had recently been commenced on suboptimal treatment (standard first-line therapy plus streptomycin) and this was suspended for ten of 26 ward days because of adverse effects.

The finding that sputum smear positivity was associated with TB transmission concurs with previous studies [5–7]. The effect of treatment on the infectiousness of TB patients is also well known [2,5], with numbers of viable bacteria falling precipitously following initiation of effective chemotherapy [34,35]. Whilst some data suggest that apparent cure of MDR TB may be achieved with first-line drugs [36,37], other studies have shown poor outcomes for such patients [38]. The current study shows how suboptimal treatment of MDR TB patients is likely to facilitate ongoing TB transmission. There is conflicting evidence concerning the relative transmissibility of MDR versus drug-susceptible TB strains [14–20]. In this study, patients with MDR TB were significantly more likely than those without MDR TB to transmit TB to guinea pigs. However, this finding should be interpreted with caution because of colinearity with suboptimal treatment (5 of 6 identified infectious MDR TB patients were on suboptimal regimens, and the other had treatment initiation delayed for 11 days whilst suitable medications were acquired). Regardless, the high relative infectiousness of inadequately treated MDR TB patients demonstrated in this study underscores the importance of prompt specific treatment guided by rapid drug-susceptibility testing, rather than restricting MDR TB testing and specific therapy to patients who survive failing empiric first-line therapy, as currently happens in most low-resource settings. It also has important implications for hospital policies that allow suboptimally treated MDR TB cases to be cared for in multi-bedded rooms.

The highly infectious nature of some of the HIV-positive MDR TB patients identified in this study has important implications for TB infection control. Administrative control measures that facilitate the rapid diagnosis, isolation, and prompt treatment of such patients are paramount. With increasing congregation of infectious and susceptible individuals not only in hospitals but also in such settings as antiretroviral therapy roll-out, HIV antenatal care, and voluntary counselling and testing facilities [39], environmental control measures are also of great importance. As the infectiousness of a TB source increases, the relative protection provided by dilutional room ventilation decreases [31]

Table 3. Determinants of Patient Infectiousness: Analysis of Infectious Versus Noninfectious Patients

Independent Variable[a]	Patient Admissions[b]		Univariate Logistic Regression[c]					Multiple Logistic Regression[d]			
	Infectious, $n = 10$	Noninfectious, $n = 39$	Simple		Clustered	Exact		Simple		Exact	
			OR	p-Value	p-Value	OR	p-Value	OR	p-Value	OR	p-Value
Sputum smear–positive	9 (90%)	20 (51%)	8.5 (1.0–74)	0.05	0.059	8.3 (1.0–394)	0.05	7.4 (0.8–70)	0.08	6.7 (0.7–335)	0.1
MDR-TB versus not MDR-TB	6 (60%)	6 (15%)	8.2 (1.8–38)	0.007	0.008	7.8 (1.4–51)	0.02	7.3 (1.4–37)	0.02	6.6 (1.1–48)	0.04
Suboptimal treatment	8 (80%)	12 (31%)	9.0 (1.7–49)	0.01	0.017	8.6 (1.4–95)	0.01	—	—	—	—
Monoresistant versus drug-susceptible TB	1/4 (25%)	8/33 (24%)	1.0 (0.1–12)	1.0	1.0	1.0 (0.02–15)	1.0	—	—	—	—
Days on ward, all	20 (12–34)	14 (7–25)	1.0 (1.0–1.1)	0.4	0.2	1.0 (1.0–1.1)	0.4	—	—	—	—
>12 d on ward	—	—	3.1 (0.6–17)	0.2	0.2	3.0 (0.5–33)	0.3	—	—	—	—
Days on ward without treatment, all	0.25 (0–6)	0 (0–2)	1.0 (0.9–1.1)	0.7	0.6	1.0 (0.9–1.1)	0.6	—	—	—	—
>2 d on ward without treatment	—	—	2.2 (0.5–9.6)	0.3	0.3	2.2 (0.4–12)	0.5	—	—	—	—
Age in years, all	31 (27–43)	31 (27–39)	1.0 (1.0–1.1)	0.4	0.4	1.0 (1.0–1.1)	0.4	—	—	—	—
>30 y of age	—	—	0.8 (0.2–3.1)	0.7	0.7	0.8 (0.2–4.0)	1.0	—	—	—	—
20–29 versus ≥40 y of age	—	—	0.8 (0.1–4.4)	0.8	0.8	0.8 (0.1–6.8)	1.0	—	—	—	—
30–39 versus ≥40 y of age	—	—	0.6 (0.1–3.6)	0.6	0.6	0.6 (0.1–5.6)	0.9	—	—	—	—
Male sex	9 (90%)	29 (74%)	3.1 (0.4–28)	0.3	0.3	3.0 (0.4–149)	0.6	—	—	—	—

[a]For categorical independent variables, the number of cases (% of total in parentheses) is shown, and for continuous independent variables, the median (IQR in parentheses) is shown. The primary analysis treated the independent variables "Days on Ward" and "Days on Ward without Treatment" and "Age" as raw, continuous variables. These primary analyses are complemented by secondary analyses (in parentheses) of each of these variables divided into categories around cutoff points chosen to maximize the likelihood of the outcome. Note that the analysis of these independent variables as continuous or categorical data gave similar results.

[b]Regression analysis was performed on putative determinants of infectiousness for the 49 ward admissions by pulmonary TB patients for which spoligotype patterns were available. Four patients accounted for the ten multiple admissions in this group. The first had MDR TB and was infectious on one admission when inadequately treated, and non-infectious on a subsequent admission when adequately treated. The second had four admissions: three were noninfectious with drug-susceptible TB that was first smear-positive and then smear-negative. The fourth admission was infectious, with acquired MDR-TB. The third patient had two noninfectious admissions separated by 4 mo with smear-positive inadequately treated monoresistant TB. The fourth patient had two noninfectious admissions separated by two months with smear-negative drug-susceptible TB.

[c]The simple univariate regressions are shown with robust clustered SE estimated (to account for repeated hospitalizations by some patients) and with exact logistic regression (to account for the sample size). Note that simple, clustered, and exact univariate logistic regressions gave similar results. ORs (95% confidence intervals) are included.

[d]Because of the sample size, only the three independent variables with clear associations with infectiousness (sputum-smear, MDR TB and suboptimal treatment) were considered for multiple regression analysis. In both simple and exact multiple logistic regression, the best model by AIC criteria included only sputum-smear positivity and MDR TB (exact multiple logistic regression AIC = 43.8). ORs (95% confidence intervals) are included.

OR, odds ratio.

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and may become inadequate at the relatively low levels of air exchange usually provided by mechanical ventilation. High rates of ventilation would be required to provide protection from the extremely infectious newly diagnosed MDR TB case observed in this study. Achieving this through mechanical means is an expensive solution for much of the world where TB is most prevalent. In contrast, well-designed natural ventilation [40] provides high ventilation rates for little cost, and furthermore is highly applicable to areas such as crowded waiting rooms where infectious, untreated TB patients are most likely to be encountered. TB infection control must be a priority in the current roll-out of enhanced HIV care, and should be carefully considered in the design and construction of any new infrastructure for such programmes.

The need for strengthened TB infection control is also highlighted by the recent outbreak of XDR TB amongst HIV-coinfected patients in South Africa; this outbreak was predominantly nosocomial and resulted in extremely high mortality [41]. The variability of infectiousness of patients demonstrated in this study highlights the usefulness of a potential test for TB infectiousness that would allow targeted isolation of the most infectious patients in the settings where isolation facilities are sparse, as is unfortunately the case in much of the world where TB is most prevalent. One patient in our study, with MDR TB, infected over half of the guinea pig colony. The development of tests that allow early identification and isolation of such patients in a clinical setting is a research priority.

There are some limitations to this study. The first is the incomplete set of spoligotyping data, with results in only 49 of 118 pulmonary TB admissions. Despite this deficiency, ten of at least 12 infectious patients were identified. It is possible that transmission occurred from other patients for whom spoligotyping was unavailable, but certain factors suggest that this is not the case. Most patients without spoligotype results had negative sputum cultures, and whilst smear-negative TB transmission occurs [8], smear-positive patients account for the majority [5–7]. In this study, 16 culture-positive patients, of whom four were smear positive, had no spoligotype result. Fortuitously, these patients were either temporally or phenotypically (drug susceptibility pattern) unrelated to the ten guinea pig clusters linked with infectious patients,

Table 4. Determinants of Patient Infectiousness: Analysis of the Degree of Infectiousness

Independent Variable	Data for All Patient Admissions $n = 49$	Univariate Descriptive Analysis			Univariate TOBIT Regression		Multivariate TOBIT Regression		
		Correlation Coefficient	p-Value	p-Value (Exact)	Predicted q	p-Value	Coefficient	p-Value	Predicted q Individual Covariate
Sputum smear-positive	29 (59%)	—	0.03	0.03	15	0.03	93	0.08	3.8
MDR-TB	12 (24%)	—	0.003	0.007	14	0.1	87	0.02	3.2
Suboptimal treatment	20 (41%)	—	0.004	0.003	21	0.08	—	—	—
Monoresistant TB	9/37 (24%)	—	0.9	0.7	1.3	0.9	—	—	—
Days on ward	15 (7.0–27)	0.13	0.4	0.4	5.2	0.4	—	—	—
>12 d on ward	—	—	0.2	0.2	13	0.2	—	—	—
Days on ward without treatment	0.0 (0.0–3.0)	0.078	0.6	0.6	8.6	0.9	—	—	—
>2 d on ward without treatment	—	—	0.3	0.2	18	0.4	4.3	0.09	0.15
Age in years	31 (27–40)	0.052	0.7	0.7	5.3	0.7	—	—	—
>30 y of age	—	—	—	—	6.5	0.6	—	—	—
20–29 versus ≥40 y of age	—	—	—	—	9.1	1	—	—	—
30–39 versus ≥40 y of age	—	—	—	—	10	0.9	—	—	—
Male sex	38 (78%)	—	0.4	0.5	11	0.2	—	—	—
Intercept	—	—	—	—	—	—	−178	0.006	0.12
Sigma	—	—	—	—	—	—	70	—	—

The infectiousness of individual patients (q, see Text S1) was tested for associations with the independent variables listed in the table. The *p*-value refers to the univariate associations assessed with the Mann-Whitney U test for categorical independent variables and the nonparametric Spearman correlation coefficient for continuous independent variables. The *p*-value is also shown for equivalent exact statistical testing. The Tobit (censored) regression analysis allows for the censoring of data from patients who did not infect any guinea pigs and who therefore had an undetectable degree of infectiousness (see Text S1). After an initial model fit for all covariates individually, the variable that entered the model based on the highest pseudo-R^2 was MDR TB. Each additional variable added would be based on the lowest *p*-value derived from likelihood ratio statistic provided that the *p*-value was < 0.2. The second variable selected was sputum-smear positivity and the third variable entered was the categorical variable of more than 2 d on the ward without treatment. No additional variables entered the model. The final model has a pseudo-R^2 of 0.10.

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excluding them as coinfectors. Indeed the two clusters of MDR TB guinea pigs with unidentified infectious sources became infected at times corresponding to the ward residency of smear-positive MDR TB patients without spoligotype results. We cannot exclude the possibility that guinea pig infections occurred from staff or visitors with TB, however all staff and visitors wore particulate respirators. It is possible that the large monoclonal outbreak observed in the guinea pigs was in fact made up of more than one strain. However, the concordant drug susceptibility data and epidemiological match with a patient on the ward at an appropriate time prior to the infections obviates the need for secondary typing of strains. The Wells-Riley model has inherent limitations [42], but these do not influence evaluations of relative infectiousness, and it allows comparison with published values of TB infectiousness calculated using the same model. The design of this study did not permit determination of the duration of patient infectiousness because of the interval of one month between skin tests, and the variability in the period required for these guinea pigs to become PPD positive following TB infection. It is possible that values for patient infectiousness are underestimates, because the entire period of a patient's hospital admission was used for the exposure duration variable in calculations, and it would normally be expected for patient infectiousness to tail off once treatment was initiated, although this would not be the case with suboptimal treatment. However, in univariate analyses patient days on the ward was not significantly associated with TB transmission. The relatively narrow age range and the small number of women amongst the patients is a further limitation of this study, because both young age and male sex have been associated with TB transmission to contacts [15,43]. Because all patients were HIV positive, our study was unable to yield evidence concerning the infectiousness of HIV-positive versus HIV-negative MDR TB patients, and this could be a future area of study using our airborne infection facility.

In conclusion, this study has demonstrated the potential of HIV-positive patients with MDR TB to be highly infectious. With the great majority of TB transmission in this study occurring from inadequately treated MDR TB patients, these results identify the importance of early drug susceptibility testing and initiation of effective chemotherapy for drug-resistant TB to prevent ongoing transmission and facilitate TB control. Furthermore, this study highlights the importance of environmental control measures to prevent airborne TB transmission in crowded health care settings, especially in areas with a high prevalence of HIV infection and drug-resistant TB, and in today's era of emerging XDR TB. HIV-positive patients with unrecognised or inadequately treated MDR TB coinfection may be highly infectious, and effective TB infection control measures are essential to prevent health care facilities from disseminating drug-resistant TB.

Supporting Information

Text S1. (A) Patient infectiousness using the Wells-Riley equation. (B) Estimating predicted degree of infectiousness, q, from Tobit (censored) regression.

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Editors' Summary

Background. Every year, more than nine million people develop tuberculosis—a contagious infection usually of the lungs—and nearly two million people die from the disease. Tuberculosis is caused by *Mycobacterium tuberculosis*. These bacteria are spread in airborne droplets when people with the disease cough or sneeze. Most people infected with *M. tuberculosis* never become ill—their immune system contains the infection. However, the bacteria remain dormant within the body and can cause tuberculosis years later if host immunity declines. The symptoms of tuberculosis include a persistent cough, weight loss, and night sweats. Diagnostic tests for the disease include chest X-rays, the tuberculin skin test, and sputum cultures (in which bacteriologists try to grow *M. tuberculosis* from mucus brought up from the lungs by coughing). Tuberculosis can usually be cured by taking several powerful antibiotics daily for several months.

Why Was This Study Done? Scientists performed definitive experiments on airborne tuberculosis transmission in the 1950s by exposing guinea pigs to the air from a tuberculosis ward. They found that a minority of patients actually transmit tuberculosis, that the infectiousness of transmitters varies greatly, and that effective antibiotic treatment decreases infectiousness. Since the 1950s, however, multidrug-resistant (MDR) and more recently extensively drug-resistant (XDR) strains of *M. tuberculosis* have become widespread. Treatment of drug-resistant tuberculosis is much more difficult than normal tuberculosis, requiring even more antibiotics, and for long periods, up to 2 years and beyond. In addition, HIV (the virus that causes AIDS) has emerged. HIV weakens the immune system so HIV-positive people are much more likely to develop active tuberculosis (and to die from the disease, which also speeds the development of HIV/AIDS) than people with a healthy immune system. Have these changes altered tuberculosis transmission between people? The answer to this question might help to optimize the control of tuberculosis infection, particularly in hospitals. In this study, the researchers investigate current patterns of tuberculosis infectiousness among HIV-positive patients by recreating the 1950s guinea pig model for tuberculosis transmission in a hospital in Lima, Perú.

What Did the Researchers Do and Find? The researchers passed all the air from an HIV–tuberculosis ward over guinea pigs housed in an animal facility on the hospital's roof. The guinea pigs were tested monthly with tuberculin skin tests, and tissues from positive animals were examined for infection with *M. tuberculosis*. Sputum was also collected daily from the patients on the ward. The researchers then used the timing of patient admissions and guinea pig infections, together with the drug susceptibility patterns and DNA fingerprints of the *M. tuberculosis* strains isolated from the animals and the patients, to identify which patients had infected which guinea pigs. Finally, they used a mathematical equation to calculate the relative infectiousness of each patient in airborne infectious units ("quanta") per hour. During the 505 study days, although 97 HIV-positive patients with tuberculosis were admitted to the ward, just ten patients were responsible for virtually all the characterized cases of tuberculosis among the guinea pigs. Six of these patients had MDR tuberculosis that had been suboptimally treated. The average patient infectiousness over the entire study period was 8.2 quanta per hour—six times greater than the average infectiousness recorded in the 1950s. Finally, the three most infectious patients (all of whom had suboptimally treated MDR tuberculosis) produced 226, 52, and 40 quanta per hour.

What Do These Findings Mean? These findings show that a few inadequately treated HIV-positive patients with MDR tuberculosis caused nearly all the tuberculosis transmission to guinea pigs during this study. They also show for the first time that tuberculosis infectiousness among HIV-positive patients is very variable. The increase in the average patient infectiousness in this study compared to that seen in the 1950s hints at the possibility that HIV infection might increase tuberculosis infectiousness. However, studies that directly compare the tuberculosis infectiousness of HIV-positive and HIV-negative patients are needed to test this possibility. More importantly, this study demonstrates the potentially high infectiousness of inadequately treated MDR TB patients and their importance in ongoing TB transmission. These findings suggest that rapid, routine testing of antibiotic susceptibility should improve tuberculosis control by ensuring that patients with MDR TB are identified and treated effectively and quickly. Finally, they re-emphasize the importance of implementing environmental control measures (for example, adequate natural or mechanical ventilation of tuberculosis wards, or crowded waiting rooms or emergency departments where tuberculosis patients may be found) to prevent airborne tuberculosis transmission in health-care facilities, particularly in areas where many patients are HIV positive and/or where MDR tuberculosis is common.

Additional Information. Please access these Web sites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.0050188.

- The US National Institute of Allergy and Infectious Diseases provides information on all aspects of tuberculosis, including multidrug-resistance tuberculosis, and on tuberculosis and HIV
- The US Centers for Disease Control and Prevention provide several fact sheets and other information resources about all aspects of tuberculosis (in English and Spanish)
- The World Health Organization's 2008 report on global tuberculosis control—surveillance, planning, financing provides a snapshot of the current state of the global tuberculosis epidemic and links to information about all aspects of tuberculosis and its control (in several languages)
- HIVInsite provides detailed information about coinfection with HIV and tuberculosis
- Avert, an international AIDS charity, also provides information about the interaction between HIV and tuberculosis
- Tuberculosis Infection-Control in the Era of Expanding HIV Care and Treatment is a report from the World Health Organization